Inheritance of Lethality on *Drosophila melanogaster*

BU-125-M James L. Baumann and W. T. Federer* December, 1960

Abstract

Conflicting evidence has accumulated in recent years concerning the possibility of lethals being produced by the process of recombination. In theory such events can be expected to occur. Observations made during laboratory experiments on the release of variability through recombination have indicated that lethals may be formed in this manner. Proving that these lethals are due to multifactor interaction by a mapping technique has not been satisfactory.

A different approach to the problem is needed. If lethals can be synthesized, then desynthesis should also be possible. Experiments have been designed to test this desynthesis approach. Furthermore, it is of great interest to see to what extent "synthetic" or multifactor lethals exist in natural populations. A sample of lethals from a population of *Drosophila melanogaster* is being tested to determine the frequency of multifactor pair lethals.

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Many heritable characteristics of biological entities are controlled by multifactor genetic systems. In recent years, much work has been done concerning the amount of variability released by recombining the components of these systems (1,2,8,9,10,11,12,13). Although mutation must be regarded as the prime source of variability, the introduction of newly arisen mutants into various genetic backgrounds allows for varying degrees of expression of the mutant. It is against these various background genotypes that selection operates on the mutant. The amount of variability released by recombination is still a moot question. The viability of an organism, having one of its chromosome pairs in homozygous condition, is the characteristic most often used for study. One method for measuring the viability of an organism is to compare the number of organisms carrying the chromosome in homozygous condition which survive to some stage of the life cycle to the number of organisms carrying the chromosome in heterozygous condition which survive to the same stage. Wallace, *et al.* (12), found that if two chromosomes having known viability characteristics were allowed to recombine with one another, the recombinant chromosomes from such an original pair, when homozygous, exhibited a range of viabilities which extended beyond that of the original chromosomes, even some lethals being produced. This occurred almost without regard to the viabilities of the original pair of chromosomes. Dobzhansky and his associates (1,2,8,10,11) found similar results in three different species of Drosophila even though all of the original chromosomes had normal viabilities. Again lethals were formed. It is important to remember that not all of these lethals which arose could be accounted for on the basis of direct mutation during the course of the experiment. Some of these lethals were apparently "synthesized" by the recombination process, bringing together two or more factors on the same chromosome which when in homozygous condition reacted together to cause lethality. These recombinational lethals have been termed "synthetic lethals" (1).

Gantner and Hildreth (4,5,6) have repeated some of this work. They also found a wide range of variability released but the lethals they obtained in this manner could be accounted for by direct mutation. Mapping tests confirmed that

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these lethals need not be considered as the result of the recombination process. Thus we see that there appears to be contradicting evidence as to whether lethals can arise through recombination.

Despite evidence to the contrary, we would expect on theoretical consideration that synthetic lethals can occur. Certain combinations of recessive laboratory mutants when on the same chromosome are not viable in the homozygous condition. Why then should not non-morphological mutants react in the same way? Furthermore, do synthetic lethals exist in natural populations?

It is to the latter of these questions to which we shall address ourselves in the investigation proposed below. We may consider two models concerning lethals; one, all lethals exist as the result of direct mutation, i.e., as single factor pair lethals, and two, that some proportion of lethals exist as the result of recombination, i.e., as multifactor pair lethals (synthetic lethals).

If we can assume that the proportion of chromosomes carrying zero lethals, the proportion carrying one-factor pair lethals, the proportion carrying two-factor pair lethals, etc., have a Poisson distribution, then with the proper technique we can estimate the terms of the Poisson.

Factor pairs involved	0	1	2 or more
Term of Poisson	e^{-m}	me^{-m}	$\sum_{2}^{\infty} \frac{m^X e^{-m}}{X!} (=1-e^{-m}-me^{-m})$

It should be possible by the technique outlined below to distinguish between one-factor pair and multifactor pair lethals. We may then estimate the Poisson parameter $\underline{m}$ and thus extend the inference to the proportion of chromosomes carrying two-factor pair lethals, three-factor pair lethals, etc. To estimate $\underline{m}$ we shall use the ratio

$$\frac{\hat{m}e^{-\hat{m}}}{1-e^{-\hat{m}}-\hat{m}e^{-\hat{m}}} = \frac{\text{no. of one-factor pair lethals}}{\text{no. of multifactor pair lethals}}$$

Present evidence (see Appendix) would seem to indicate that higher order lethals (those involving say 4 or more factor pairs) will probably be rare.

With proper experimental technique the relative proportions p_0, p_1, and $\sum_{i=2}^{\infty} p_i$ can be estimated. There are probably at least 400 loci on the 2nd chromosome of D. melanogaster. The lethals occurring on this chromosome will serve as our

material for study. It is planned to investigate the pattern of inheritance of K=100 non-allelic lethals.

The experimental approach to the problem follows:

The crosses to be made are:

G1. $5\,[\,5CyL/1^n\ \male\male \times 5\ \tfrac{+}{+}\ \female\female\,]$

Experimental ↙ ↘ Control

G2. $5\,[\,5\ \tfrac{+}{1^n}\ \female\female \times 5CyL/1^n\ \male\male\,]$ $\qquad$ $5\,[\,5\ \tfrac{+}{1^n}\ \male\male \times 5CyL/1^n\ \female\female\,]$

Transfer G2 crosses once to fresh bottles on 4th day after mating to give 10 bottles total in both experimental and control sets.

G3. Count the 10 bottles on the 12th, 15th, and 18th day after a given bottle has been started. Expected results are given below.

gametes	$\frac{1}{2}$ CyL	$\frac{1}{2}$ 1
$\frac{1-r}{2}$ "+"	$\frac{1-r}{4}$	$\frac{1-r}{4}$
$\frac{1-r}{2}$ 1	$\frac{1-r}{4}$	$\frac{1-r}{4}$
$\frac{r}{2}$ 1-"+"	$\frac{r}{4}$	$\frac{r}{4}$
$\frac{r}{2}$ "+"-1	$\frac{r}{4}$	$\frac{r}{4}$

r = frequency of recombinant chromosomes.

r cannot exceed 0.5 assuming certain cytological conditions.

frequency of "+ type" phenotype expected is $\frac{1+r}{3+r}$

If r=0 then frequency of "+ type" is 0.33 and we presumably have a one-factor pair lethal. If r = some value ($0.00 < r \leq 0.50$) then we have a multifactor pair lethal.

The thinking for the above crosses is as follows: If the lethal is the result of multiple-factor pairs then we should be able to separate these factor pairs through recombination with non-lethal bearing chromosomes to produce non-lethal chromosomes. These recombinant chromosomes can be tested against the original lethal chromosome. If the lethal complex has been broken up then the

recombinant chromosome and the lethal will form a viable heterozygote pair. If the complex remains intact then, when testing against the parent lethal, we will have a homozygous pair of chromosomes which is lethal. We will expect an excess of viable pairs of chromosomes if the lethal follows the multifactor pair model. The frequency of viable pairs will be $\frac{1+r}{3+r}$ (see above). Recombinant chromosomes are obtained from the females heterozygous for the lethal and a chromosome of normal viability. Males of similar constitution serve as controls, since no recombination occurs in D. melanogaster males.

Our ability to detect closely linked multifactor lethals is limited by the practical consideration of the number of observations which must be made to detect a significant difference between the experimental and control runs. If $\sqrt{\frac{pq}{n}} = S.E._{mean}$, where p = frequency of "+" type in control and n is number of observations (flies), then we can estimate how many observations are required to give a certain resolution (i.e., the value r) for the experiment.

p=0.33 and n=2000 will give r=0.10. At the present time it has been decided that this is the maximum number of observations which can be made and still keep the project small enough to be handled by one person.

The X^2 test will be used to test the difference between the experimental and control observations.

The above experiments to test the model assume that a random sample of 2nd chromosome lethals has been obtained without knowledge of their mode of inheritance.

This experiment will be conducted by James L. Baumann. Some work has already been done on this experiment while Mr. Baumann was at the University of Rochester, New York. These results are attached. No satisfactory conclusions can yet be drawn from these data.

Appendix A

Table I shows results of experiments conducted to date. Data columns show total number of flies of a phenotypic class in all bottles counted for that experiment. All results except L56III, L183II, and JL2II are based on totals of ten bottles counted. The latter are based on twenty bottles counted.

The X^2 test is used to compare experimentals and controls. It will be noted that six of the X^2's plus the grand total X^2 are significant at $p=0.05$. However, in only one of these cases, L183I, is the difference due to the results for the experimentals being higher than for the controls. The other five cases are disturbing, and further experiments must be planned to investigate these results.

Table I

Results of Desynthesis Experiments on Synthetic Lethals of Second Chromosome of *Drosophila melanogaster*

Chromosome	Experimental Cross			Control Cross			χ^2
	CyL Flies	"+" Flies	Total Flies Counted	CyL Flies	"+" Flies	Total Flies Counted	
L17	824	507 38.09 *	1331	847	697 45.14	1544	14.66
L25	3595	2080 36.65	5675	1186	641 35.08	1827	1.47
L28	3146	2042 39.36	5188	469	306 39.48	775	0.004
L56I	1203	812 40.29	2015	335	199 37.26	534	1.62
L56II	1443	847 36.99	2290	1398	773 35.61	2171	0.92
L56III	2511	1302 34.14	3813	2105	1150 35.87	3255	1.08
L56(I+II+III)	5157	2961 36.47	8118	3838	2122 35.60	5960	1.125
L60	1370	602 30.53	1972	1374	819 37.35	2193	21.3
L61	2816	1628 36.63	4444	376	311 45.26	687	18.84
L82	1797	1117 38.33	2914	393	254 39.25	647	0.19
L85	1687	914 35.14	2601	1806	1037 36.47	2843	1.05
L94	2443	1343 35.47	3786	1998	1143 36.39	3141	0.62
L174	1893	1008 34.75	2901	1556	829 34.76	2385	-
L183I	1501	1233 45.10	2734	1150	798 40.96	1948	7.91
L183II	1886	1241 39.96	3107	1976	1416 41.74	3392	2.19
L183(I+II)	3367	2474 42.35	5841	3126	2214 41.46	5340	.91
L222	1829	888 32.68	2717	1614	886 35.44	2500	4.41
JL2I	1405	992 41.38	2397	1148	728 38.80	1876	2.91
JL2II	2006	1318 39.65	3324	2172	1418 39.49	3590	0.016
JL2(I+II)	3411	2310 40.37	5721	3320	2146 39.26	5466	1.45
JL56	1258	829 39.72	2087	1153	904 43.95	2057	7.60
JL54	1881	1031 35.40	2912	2137	1224 36.42	3361	0.69
JL50	1868	1109 37.25	2977	1791	1157 39.24	2948	2.49
Grand Total	38342	22843 37.33	61185	26984	16690 38.21	43674	8.39

* Percent wild type of total

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